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Ethno-botanical and Toxicological comparison on varieties of *Vatsanabha* (*Aconitum ferox* Wall.) available in Nepal.

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ABSTRACT

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Introduction: The root of *Vatsanabha* (*Aconitum ferox* Wall.) is considered the most virulent of vegetable poisons. Due to this reason, it is highly exploited and traded in India. The Medicinal and Aromatic Plants Database of Nepal (MAPDON) has reported its distribution at Subalpine zone (3000-4000 meter/10000-14000 ft). *Vatsanabha* (synonyms *Bikha*, *Bisha*) is mentioned in different Ayurveda texts as *Mahavisha* (~highly toxic in nature). Its *Kanda* (root tuber) is used in the formulation for curing ailments. Acute Oral Toxicity study found a lethal dose of aconitine in mice to be 1.8 mg/kg. This survey study was conducted to explore the ethnomedicinal importance and to compare the toxicity of those tuberous roots which are available geographically in Nepal.

Methods: Field survey was carried out and interviewed with the indigenous people, traditional practitioners and herbal traders. The Sample collected was evaluated for Acute Oral Toxicity (AOT) on Wistar Albino rat as per Organisation for Economic Co-operation and Development (OECD) 425 Guidelines. All the rats were dosed per orally at constant dose-volume fixed as per AOT Software Program. The toxicological parameters were entered and evaluated on AOT Performa for Physical and Behavioural Study.

Results: Local people and indigenous practitioners were aware of *Vatsanabha*, its uses and toxicity. Traditional practitioners at higher altitude collect, store and process the roots for preparing formulations. The root powder is used for the treatment of joint pain, fever and against microbial contamination. The lethal doses of the Naradevi, Shivapuri and Dolpa samples were observed to be 29.57 mg/kg (C.L. 17.5 - 55), >2000mg/kg and 29.57 mg/kg (C.L. 17.5 to 55) respectively.

Conclusions: Majority of the traditional practitioners ethnic groups inhabitant of Nepal in higher altitude use *Vatsanabha* to prepare their own different formulations for curing the illness. The lethal doses obtained from Wistar albino rat experimentation was 29.57 mg/kg and 17.5mg/kg wt. This study recommends the quantitative analytical study of *Vatsanabha* to support the toxicological analysis.

Keywords: *Aconitum ferox*; AOT Test; Higher altitude; *Vatsanabha*..

INTRODUCTION

The 'ferox' is a Latin word, which means "fierce, ferocious, very prickly, very thorny, strong or wild". Generally, the term 'ferox' is used to refer to very spiny plants. However, it possibly refers to the ferociously poisonous nature of the roots of the plant.¹ It is to be noted that *A. ferox* is considered as one of the most poisonous plants in the world.

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This species is endemic to the Himalayas. *Aconitum ferox* is also known as Indian Aconite. It is commonly known as Himalayan Monkshood (Monk's hood). The root of this plant is considered as most virulent of vegetable poisons. Due to this reason, it is highly exploited and traded in India. The species has been included in the Red Data Book of Indian Plants and later 'Negative List of Exports' in 1998 by Government of India, which emphasizes its rarity in the wild and immediate action for its conservation.²

India receives its supply mostly from Nepal.³ *Aconitum ferox*, in the nineteenth century, there was a thriving trade in the root tubers of *ferox*, which was bought from Lhasa via Le (Mustang). The geographical areas of India and Nepal were considered for the study. *Visha Dharana* (wearing Aconite) is one of the prophylactic measures for *Visama Jwara*..

Acharya Charaka includes *Vatsanabha* in *Sthavara Visha*.⁴ *Acharya Sushruta* mentioned *Vatsanabha* under thirteen varieties of '*Kanda Visha*'.⁵ First internal administration of *Vatsanabha* as *Prativisha* to treat patients of poisoning was described by *Acharya Vagbhata*. *Vatsanabha* is of four types according to race-⁶ 1. *Bramhana*: with hairs, white. 2. *Kshatriya*: red, 3. *Vaishya*: yellow in colour, sweet in taste and 4. *Shudra*: black. According to properties of poisons,⁷ *Brahmana* variety of toxins is to be used in diseases; the *Kshatriya* variety to be given to a patient who has swallowed some poison; the *Vaishya* variety is to be used in conditions of minor importance, and the *Shudra* variety to be given to a man who has been bitten by a poisonous snake.⁸ The traditional and cultural expression that form ethnobotanical importance has not been evaluated with respect to *Vatsanabha* in Nepal.

International Union for Conservation of Nature (IUCN), Nepal has enlisted three varieties of *Aconitum* in its availability with terminology *Bikha* for *Aconitum ferox* and *Aconitum ferox spicatum*. And *Bikhama* for *Aconitum Bisma* (Buch-Ham) and *Aconitum palmatum* as per the National Register of Medicinal and Aromatic Plants as commercially threatened.^{9,10} The study was carried on to review those varieties of *Vatsanabha* based on the reported geographical areas mapping. The Medicinal and Aromatic Plants Database of Nepal (MAPDON) has

reported its distribution at Sub-alpine Zone (3000-4000 meter/10000-14000 ft),¹¹ at Himalaya regions, areas around Shey Phoksundo, Dolpa, Nepal,¹² and Shivapuri, Kathmandu, Nepal (as per Herbarium at National Herbarium & Plant Laboratories, Kathmandu, Nepal by Tirtha Bahadur Shrestha in 1992 A.D.). Being focussed on the ethnobotanical and toxicological review of those collected samples, the study was undertaken.

METHOD

The Survey was done on different parts of Nepal. An observational study based on the judgmental sampling method was undertaken.

Survey study on Mustang, Nepal was done in November end 2018- interaction with representatives of Himalaya Amchi Association (Figure 1.b), Amchi at *Lomanthang* (Upper Mustang), Jharkot and Jomsom, person engaged in Herbal Medicines - Shree Jharkot Traditional Medical Center, District Ayurveda Center, Mustang and local people. The first survey on Shivapuri was carried out in December 2018 A.D. followed by March 2019 A.D. and November 2019 A.D., where the local traders of herbal medicines and indigenous people of the areas were interviewed. Survey on Naradevi market (Figure 1.a) and Dolpa was carried out in April 2019 by obtaining permission from the Department of Plant Resources, Thapathali, Ministry of Forest, Government of Nepal.

Access to the field survey was extended to conservation areas after obtaining permission from Shivapuri-Nagarjun National park, Kathmandu, Shey-Phoksundo National Park, Dolpa, Annapurna Conservation Area Project (ACAP), Pokhara under the Department of National Conservation and Wildlife Protection, Ministry of Forest and Environment, Government of Nepal. Authentication was done by the National Herbarium and Plant Laboratories Godavari-3. Lalitpur, Bagmati 44700.

The Experimental Study was approved from the Institutional Animal Ethical Committee (SDM Center for Research in Ayurveda and Allied Sciences, Kuthpady, Lakshminarayana Nagar, Udupi 574118) with SDMCRA/IAECI/AG-14.

Tuberous root for the experimental study was collected by direct field visit method to study area (Figure 2). Quadrat sampling technique was used in order to obtain uniformity

of the sample. Geography selected for sample collection belongs to Naradevi, Dolpa district and Shivapuri. The commercial sample was Taken from Naradevi, Kathmandu, Nepal - significant trade area of Nepal for herbal and indigenous products. The Sample for group 2 was collected from the Shivapuri (height of 2800 m) and group 3 from Dolpa (near Shey Phoksundo) at the height of 3615 m (29.2050 N, 82.9550 E).

The Sample was transported to the Animal Experimental Lab for the toxicological study. Due to unavailability of the experimental animal facility as per the study design, the experimentation was carried out at SDM Center for Research in Ayurveda and Allied Sciences, Karnataka, India. Permission has been granted from National Ayurveda Research and Training Centre, Kathmandu, Nepal with a disclaimer of intellectual property rights reserved to Nepal and traditional knowledge bearers of Nepal.

The three samples from Naradevi, Dolpa and Shivapuri were used as intervention on Wistar albino rats which was brought from Animal House attached to the SDM Center for Research in Ayurveda and Allied Sciences. A minimum of 5 healthy either sex of body weight 150-250g rats were selected according to OECD 425 guideline.¹³

All the selected rats were kept under acclimatisation for seven days before dosing. All animals were marked with the saturated picric acid solution in water for proper

identification. The marking within the cages and dosing was done as per body weight of the rat. The prepared test sample (*Vatsanabha* root powder) was made into suspension in water with suitable concentration at the rate of 1ml/100 g weight of rat. All the rats were dosed (in accordance to identification tag) per orally at constant dose-volume fixed as per AOT Software. Dose fixation was done in step-up-down flow within 2000 mg/kg, 175 mg/kg, 55 mg/kg, 17.5 mg/kg, 5.5 mg/kg as per the software.

Examination of physical and behavioural changes:

After the animals dosing, they were observed for 4 hours. The cage side observation was carefully done without disturbing the animal's attention and at the end of the every hour the animal was individually exposed to open arena for recording the behavioural changes like decreased or increased motor activity, catatonia, spasticity, opisthotonus, convulsion, straub's reaction, muscle spasm, hyperesthesia, muscle relaxation, anaesthesia, arching and rolling, lacrimation, salivation, diarrhoea, writhing, mode of respiration, changes in skin colour, etc, CNS depression- hypo activity, passivity, relaxation, ataxia, narcosis, etc. The data is entered on Performa for assessing Gross Behaviour for reviewing the changes. All the animals were observed at 1, 2, 3, 4, 24, 48 hrs after dosing and thereafter daily once for mortality during the entire period of study (i.e 14 days).

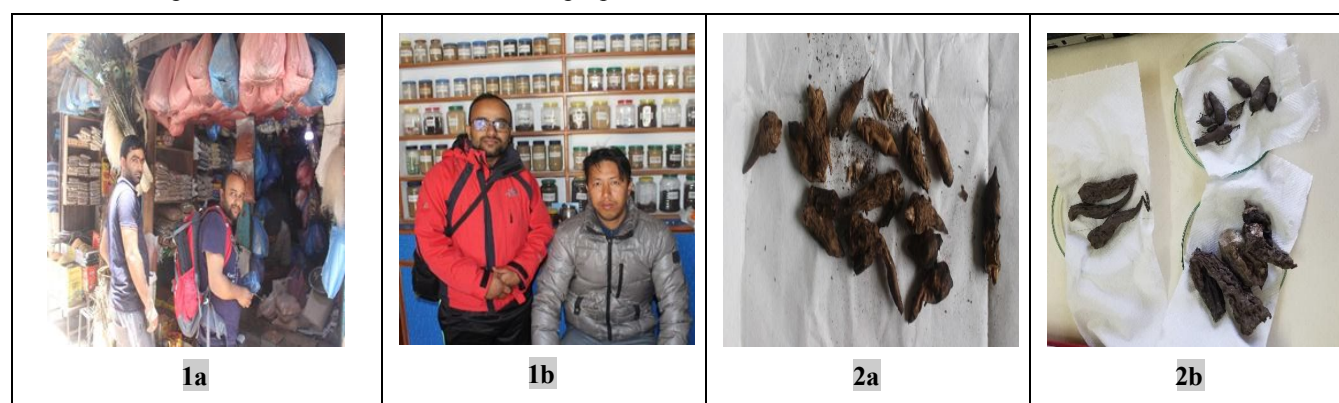


Figure 1: Photographs of Ethnobotanical survey during study. **Fig. 1.a.** Market survey on the Naradevi and collection of market samples. **Fig. 2.b** Interview with local practitioners (Amchi) of Mustang region.

Figure 2: Photographs of the Experimental sample for the Toxicological study. **Fig. 2.a** Samples from Dolpa. The sample was provided by Traditional Practitioners. **Fig. 2.b.** Samples from three regions: Naradevi, Dolpa and Shivapuri.

RESULTS

Vatsanabha is identified by the local people and indigenous practitioners with synonyms of *Bikha*, *Bisha*. The Herbarium/root tuber of the sample was authenticated as *A. ferox* var *spicatum* for Naradevi and Dolpa and *A. ferox* of Shivapuri.

Dolpa, Nepal

Lama, indigenous practitioners belonging to Buddhist religion, and *Amchi* are active health practitioners at Dolpa region. They collect herbs locally available and uses for curing local people. They collect the *Vatsanabha* for the manufacturing of their own local medicinal products. Such medicinal products are not traded nor commercially marketed. In the Tibetan language, it is called as *Dhhuka*, which is called as the poison in the *Gurung* and *Tibetan* language. The local people easily identify it, and they even don't touch it. They reported that the yak newly transported to the high altitude height usually died due to the grazing in those areas.

Mustang, Nepal.

The local name of the poisonous plants is *Bhongaar*, *Kenduk*, *Ghona*. Traditional practitioners collect fresh *Vatsanabha* from the areas of Dolpa. They prepare the medicines for the ailment of joint pain and fever. Practitioners travel to the areas during the collection season and collect them in huge amounts and store them safely. For safety, they manage their store room as a secret place and don't let their family members enter the storage room.

Naradevi, Nepal.

Naradevi market is one of the biggest markets of Nepal, where we can find most of the herbal raw materials. They collect the raw materials of *Vatsanabha* from different regions of Nepal. The raw materials are sold to the Pharmacy and Pharmaceuticals for the preparation of medicines. Shopkeeper believes in *Ativisa* (*Aconitum heterophyllum*) the antidote of *Vatsanabha*.

Experimental:

Experimental evaluation of acute toxicity study of *Vatsanabha* root powder as per the guidelines of OECD 425 Acute Oral Test. The Observation (Table 1, Table 2, Table 3) was made on *Vatsanabha* rhizome powder dosing as per the protocol and verified with AOT software. Blanching, diarrhoea, nasal secretion and decreased motor activity were observed on the rats due to toxicity. The rat died due to respiratory distress and convulsions.

The LD50 value of the Naradevi sample was found to be 29.57 mg/kg with a confidence limit of 17.5 to 55 mg/kg. The data generated are derived from AOT statistical program (AOT425StatPGm, Version 1.0, May 2001, Westat (Under Contract to EPA)).¹⁴ The LD50 value of the Dolpa sample was found to be 17.5mg/kg with 95% CI. Confidence interval is 5.494 to 59.5. And the LD50 value of Shivapuri sample was found to be >2000mg/kg.

Table 1. Dosing and Observation after dosing of Group Naradevi(Group 1).

Animal no	Identification of animals	Desired dose (according to AOT software- mg/kg body wt)	Bodyweight In grams	Calculated dose (ml)	Mortality
1.	Head	175	225	2.25	Died in 2 hours
2.	Neck	55	250	2.5	Died in 1 hour 30 minutes.
3.	Body	17.5	201	2.0	Rat survived.
4.	Tail	55	188	1.8	Died within 1 hour 20minutes
5.	No marks	17.5	190	1.9	Rat survived.
6.	Head and neck	5	200	2.0	Rat died at 2 hour
7.	Forelimb	17.5	179	1.8	Rat survived.

Table 2. Dosing and Observation after dosing of Group Shivapuri (Group 2).

Animal no	Identification of animals	Desired dose (according to AOT software- mg/kg body wt)	Bodyweight In grams	Calculated dose (ml)	Mortality
1.	Head	175	302	3.02	Rat survived.
2.	Neck	175	180	1.80	Rat survived.
3.	Body	550	210	2.10	Rat survived.
4.	Tail	550	240	2.40	Rat survived.
5.	No marks	2000	190	1.90	Rat survived.

Table 3. Dosing and Observation after dosing of Group Sikkim (Group 3).

Animal no	Identification of animals	Desired dose (according to AOT software- mg/kg body wt)	Body weight In grams	Calculated dose (ml)	Mortality
1.	Head	175	165	1.65	Rat died in 1 hour
2.	Neck	55	164	1.64	Rat died in 1.5 hours.
3.	Body	17.5	187	1.87	Rat survived.
4.	Tail	55	160	1.60	Rat died within 2.4 hours.
5.	No marks	17.5	195	1.95	Rat died after 6 hours
6.	Head and neck	5.5	227	2.27	Rat died in 1.5 hours.
7.	Forelimb	17.5	179	1.79	Rat survived..

DISCUSSION

There are not many differences in the botanical uses of the *Vatsanabha* among the ethnicity. The indigenous practitioners of the higher altitude process it to develop formulations for curing the ailments like fever, cough/cold, body ache, diarrhoea, stomach disorder and high blood pressure.^{15,16} The lower altitude local people aren't so conscious about its importance. Shivapuri is the nearby city of Kathmandu, none of the local dwellers were aware of it except traders and herbal experts of the area. It is famous for medicinal uses in the higher altitude where people depend and believe in traditional practice and medicine.

Identification of the *Vatsanabha* was based on the knowledge of the tribal people, local traditional practitioners. It is mentioned in *Charaka Samhita* that goat herders, sheep herders, cow herders and dwellers are acquainted with names and forms/ identification of various medicinal herbs and plants.¹⁷

Seed based multiplication is the most effective, practical and convenient means for most of the species.¹⁸ But, in Nepal seed propagation and cultivation is not in practice. Nepal being the natural hub of *Vatsanabha*, such practices are not observed till date.

Identification is complicated due to numerous species identical in appearance and needs utmost precaution during the collection procedure.¹⁹ Root tubers of *Vatsanabha* should be dug out after its fruits have ripened. The roots should be of *Nava* (newly grown), *Guru* (~heavy), *Sthula* (~thick), *Snigdha* (~unctuous), and fully developed. The roots should be devoid of infestation by worms and insects. It should be freshly collected after the fruiting season.²⁰ This drug will not be most potent during the winter season or during *Vasanta ritu*. Therefore, as well-grown plants, *Vatsanabha* should be uprooted during the winter season or during *Vasanta ritu* (late September) and the rhizomes are collected, washed, dried and stored for further pharmaceutical use. The Aconitum alkaloid contents can vary with the species, place of origin, time of harvest, and most important the

method and adequacy of the processing.²¹ This consideration is important for quality assurance of experimentation of natural samples. This could be relevant factors for the result of Shivapuri sample, lethal dose more than 2000 mg/kg. Even, the confounding factors can contribute to it, unless and until the higher quantitative analytical data have their relevance.

The *A. ferox* root of this plant contains 'pseudaconitine', which is considered as a highly toxic alkaloid and it is biologically 1.5 times more active than aconitine, an alkaloid reported from the *European Aconitum*, *A. napellus* L.²² *A. ferox* var *spicatum* is less toxic than *A. ferox* in nature. Even samples of Dolpa and Naradevi belonging to *A. ferox* var. *spicatum* was observed in variation in toxicity (Dolpa more toxic to Naradevi). Dolpa region is the higher altitude region of Nepal, environmental stress factor promotes alkaloidal constitutions - has more relevance.

Toxicological studies have demonstrated that the toxicity of diester-alkaloids is almost the same with LD50 values of about 0.08 mg/kg body weight. At the same time, the hydrolysed monoester alkaloids show much lower toxicity (LD50 - 24 mg/kg) in rats.²³ 2 g of the root, 250 mg of the extract, 25 drops of the tincture or 2-6 mg of the alkaloids are proved to be fatal. The average fatal period is about six hours.²⁴ Y. Xie et.al found LD50 of Aconitine in mice to be 1.8 mg/kg.²⁵

Incidence of medicolegal poisoning usually is caused due to the intentional mixture of the *Aconitum* powder or *Aconitum* rhizome tincture in the food dishes or accidental ingestion due to the same appearance with the horseradish, which is edible.²⁶ The dose and toxicity are relevant in case of the source of the experimentation, and the poisoning form is identical. This study demonstrates the LD50 of the *Vatsanabha* roots to be 5.494 to 59.5 mg/kg wt. (rat dose). The gross behavioural and physical changes: blanching, diarrhoea, nasal secretion and decreased motor activity were observed in all the three groups due to toxicity. As a new idea to study further, Shivapuri sample is a perspective for the study into the depth of qualitative and quantitative analytical study to precisely explain the nature of toxicity. Moreover, the non-alkaloidal constituents and their biological properties and their taxonomic significance for *Aconitum* plants can facilitate future research.

Limitation of the study:

This study result doesn't confirm the non-viability of the toxicological factor of Shivapuri sample. The active ingredients investigation can assist in tracing the causes behind those confounding causes. The study demonstrates the finding based on the AOT study. Observation of subacute toxicity and chronic toxicity testing can only validate the toxicology nature of the Sample. Also, Since study was conducted in manually collected samples, Findings may not be equivalent with aconite being used for trading purpose and preparation of Ayurvedic formulations.

CONCLUSIONS

Majority of the traditional practitioner's ethnic groups inhabit Nepal in higher altitude uses *Vatsanabha* to prepare their formulation for curing the illness. The lethal dose obtained from albino rat experimentation is 29.57 mg/kg and 17.5mg/kg wt. The species, place of origin, time of collection, the method and adequacy of the processing has to be considered for toxicological analysis. Uniform evaluation of the safety profile based on evidence of ethnobotanical knowledge of toxic medicinal plants, can only assure good production of Ayurveda and related drugs. This study recommends the quantitative analytical study of *Vatsanabha* to support the toxicological analysis.

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